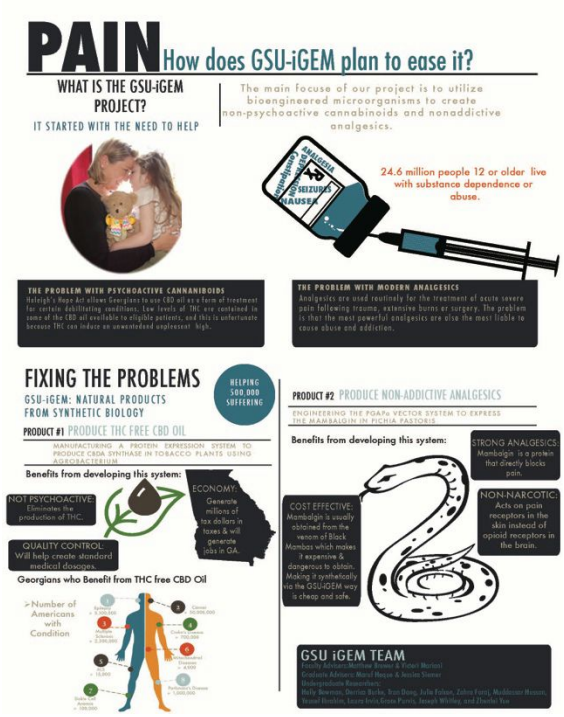
09/02/15
Finished Legislative Handout



09/01/15
Completed an enzymatic digestion, will do a ligation of this product and work on plants in late September.
08/31/15

HRP samples were digested by Yousef.

08/21/15

Purify PCR HRP Product
Laura did a purification of the PCR product on samples 2,3,5 out of the six PCR samples.
PCR Cleanup, dimers removal Protocol

1. Adjust the volume of the reaction mixture to 200 uL with water (nuclease –free or TE buffer)
2. Add 100 uL of Binding Buffer. Mix thoroughly by pipetting.
3. Add 300 uL of ethanol (96-100%) and mix by pipetting
4. Transfer the mixture to the DNA Purification Micro Column preassembled with a collection tube. Centrifuge the column for 30-60 seconds at 14,000 x g. Discard the flow through. Place the DNA Purification Micro Column back into the collection tube.
    a. If DNA fragment is ≥ 10 kb centrifuge the column for 2 minutes at 14,000x g.
    b. Close the bag with DNA Purification Micro Columns tightly after each use!

5. Add 200 uL of Prewash Buffer (supplemented with ethanol, see p. 3) to the DNA Purification Micro Column and centrifuge for 30-60 seconds at 14,000 x g. Discard the flow-through and place the purification column back into the collection tube
6. Add 700 uL of Wash Buffer to the DNA Purification Micro-column and centrifuge for 30-60 seconds at 14,000 x g. Discard the flow-through and place the purification column back into the collection tube.
7. Repeat step 6.
8. Centrifuge the empty tube DNA Purification Micro Column for an additional 1 minute at 14,000 x g to completely remove residual Wash Buffer.
9. Transfer the DNA Purification Micro Column into a clean 1.5 mL micro centrifuge tube.
10. Add 10 uL of Elution Buffer to the center of the DNA Purification Micro Column membrane. Centrifuge for 1 minute at 14,000 x g to elute DNA
11. Discard the purification column and store the purified DNA at -20°C.

08/10/2015

Ligate
promoter: BBa_J04500
RBSJ61101 laci promoter Part R0011
Completed a PCR on HRP
1.PCR HRP construct

1. Thaw primer solutions and template nucleic acid. Keep on ice after complete thawing, and mix thoroughly before use.
2. Thaw Taq PCR Master Mix and mix by vortexing briefly to avoid localized differences in salt concentration.
3. Prepare a reaction mix according to Table 1.
    a. NOTE: The reaction mix typically contains all the necessary components.

Reaction setup for six reactions.
Tube 1:
Total Reaction Volume 300 uL
Taq PCR Master mix 150 uL
Primer-IDT-GBlock R:12 uL
Primer-IDT-Gblock F: 12 uL
Template DNA: 24 auL
Deionized water: 102 uL

4. Mix the reaction mix gently but thoroughly, for example by pipetting up and down a few times. Dispense appropriate volumes into PCR tubes or the wells of a PCR plate.
5. Add template DNA to the individual PCR tubes or wells containing the reaction mix. For RT-PCR, add an aliquot from the reverse transcriptase reaction. The volume added should not exceed 10% of the final PCR volume.
6. Program the thermal cycler according to the manufacturer's instructions.

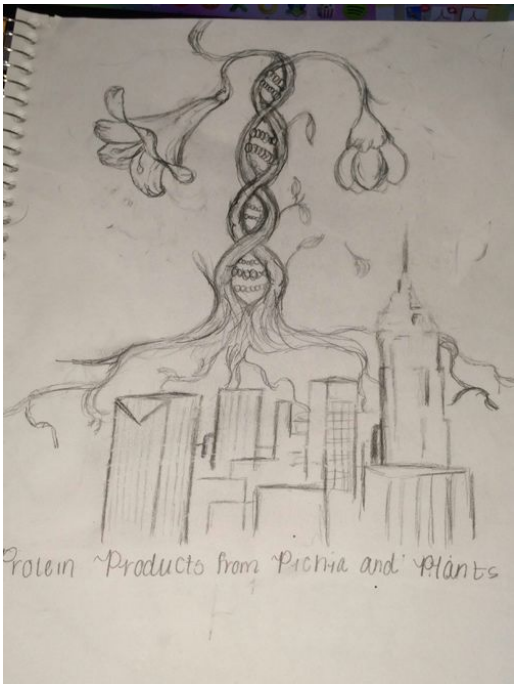
08/07/15

Team looked over the draft of the abstract and fixed it. Here is the final product.

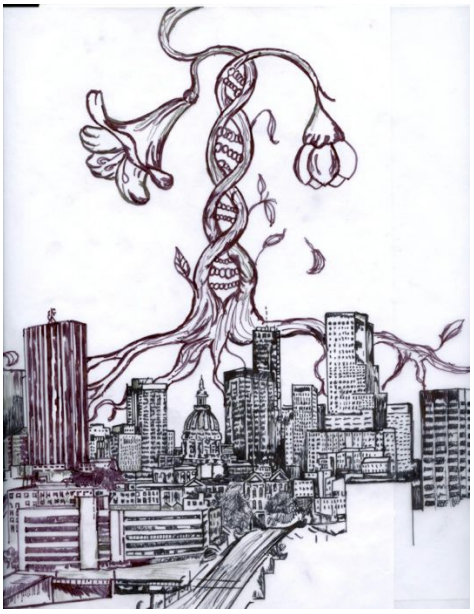
Protein Products from Plants and Pichia: Novel Manufacturing of Analgesics and Cannabinoids
Cannabinoids and opiates are widely used classes of pharmaceuticals; unfortunately, these drugs have strong psychoactive effects or can be addictive. Our project consists of two ideas, both revolving around utilizing bioengineered microorganisms to create non-psychoactive cannabinoids and non-addictive analgesics. To achieve this we developed two projects: (1) Manufacturing a protein expression system to produce CBDA synthase in tobacco plants using agrobacterium, (2) Engineering the pGAPα vector system to express the mambalgin in *Pichia Pastoris* as a continuation of the 2014 GSU iGEM project. Simultaneously, we developed a proof of concept using horseradish peroxidase. By the end of this project we hope to have produced a synthetic biological system to manufacture pharmaceutical alternatives for patients that suffer from diseases such as epilepsy, cancer, or chro

08/06/15
Finished the abstract and sent out an email to the team with a write up of the abstract. Here is a consolidation of the two abstracts that Yousef and I created.

Title: Micro-Factories: Where New Analgesics' and Phytocannabinoids are forged!
Project Abstract
For years' cannabinoids and opiates were used to create pharmaceuticals; unfortunately, the drug derivatives that have been created can have strong psychoactive effects or be addictive. Thus our project consists of two ideas; both revolving around the interconnected theme of utilizing bioengineered microorganisms to create non-psychoactive cannabinoids and non-addictive pain relievers. To achieve this two itemized projects were conducted: (1) Creating non-psychoactive cannabidiol(CBD) by transforming the plasmid containing CBDA synthase cDNA into agrobacterium and then, growing it in tobacco plants (2) Engineering the pGAPα vector system to express the mambalgin protein in pichia yeast and constructed new mambalgin plasmids that can be transformed into E.coli cells. And, this is a continuation of the 2014 GSU-iGEM project to create a powerful non-addictive analgesic. By the end of this project we hope to have produced pharmaceuticals alternatives for patients that suffer from diseases such as epilepsy, cancer, or chronic severe pain.
Also, put in to the sketch and made it look more like the city of Atlanta. So sketch went from this



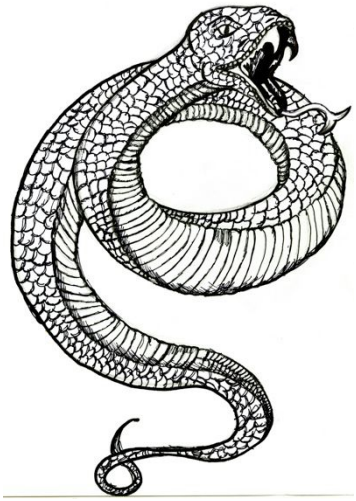
to this



08/05/15
Yousef and I have started working on the project abstract to submit to iGEM. We each have come up with individual project descriptions. I am working on consolidating the two abstracts that Yousef and I have come up with.

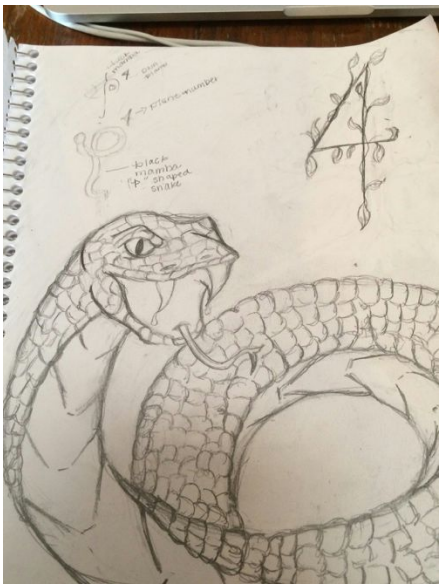
07/22/15
Started working on the Legislative Handout for a legislative outreach.
07/17/15
Sent Andrea a cleaned up version of the snake sketch.
Working steadly through the legislative handout

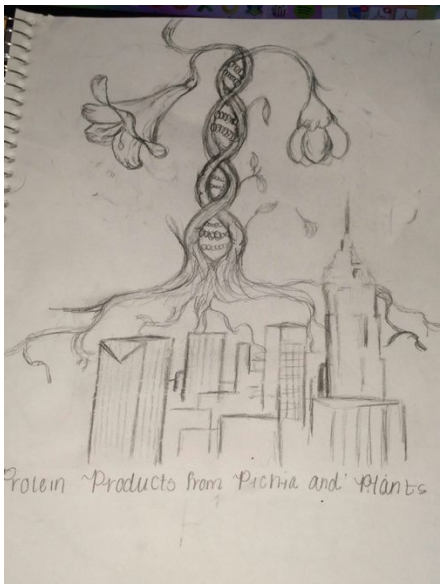
07/12/15
Finished a legislative handout
07/06/15



07/02/15
Met with Andrea and discussed some collateral things to be designed to brand the research project. Discussed the creation of: t-shirts, stickers, poster templates, how to incorporate the sketches into the website design, and the design of a letterhead for the iGEM team.

07/01/15
Andrea responded to email planning to meet at Ebrik and to go over sketches.
In contact with Kylie Bucalo from the botanical gardens about the an iGEM outreach program, working with Kylie to perfect a tissue culture protocol.
Called Mike Wenzel, got a response he is forwarding my information to Kylie Bucalo who will be point for GSU-iGEM at the botanical gardens. Decided not to wait for Kylie to send a response email about the botanical gardens. Instead called Kylie and discussed a day to come tour the tissue culture, decided to tour the lab on 07/13/15, and discussed what to view and how Kylie could assist our team.
06/30/15
Emailed Andrea preliminary sketches of the iGEM logo, after the team decided on a name for the research project this year.





06/26/15
Sent an email to Mike Wenzel at the botanical gardens in a hope to tour the tissue culture lab.

March 26, 2015
Worked on the Ethics and Careers Poster for exploration expo